Marine Environmental Research 136 (2018) 78–88

Contents lists available at ScienceDirect

Marine Environmental Research

journal homepage: www.elsevier.com/locate/marenvrev

Impact of ocean acidification and warming on the productivity of a rock pool community

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ARTICLE INFO

Keywords:
Rock pool
Acidification
Increased temperature
Fleshy algae
Coralline algae
Grazers
Primary production
Species interactions

ABSTRACT

This study examined experimentally the combined effect of ocean acidification and warming on the productivity of rock pool multi-specific assemblages, composed of coralline algae, fleshy algae, and grazers. Natural rock pool communities experience high environmental fluctuations. This may confer physiological advantage to rock pool communities when facing predicted acidification and warming. The effect of ocean acidification and warming have been assessed at both individual and assemblage level to examine the importance of species interactions in the response of assemblages. We hypothesized that rock pool assemblages have physiological advantage when facing predicted ocean acidification and warming. Species exhibited species-specific responses to increased temperature and pCO_2. Increased temperature and pCO_2 have no effect on assemblage photosynthesis, which was mostly influenced by fleshy algal primary production. The response of coralline algae to ocean acidification and warming depended on the season, which evidenced the importance of physiological adaptations to their environment in their response to climate change. We suggest that rock pool assemblages are relatively robust to changes in temperature and pCO_2, in terms of primary production.

1. Introduction

Anthropogenic emissions of carbon dioxide (CO_2) are responsible for an increase in ocean temperature and an alteration of ocean chemistry through a rapid decrease in seawater pH (Gattuso et al., 2015). If current emissions are maintained, the sea surface temperature may rise 2.7 °C and the pH decline 0.33 units by the end of the century according to the scenario RCP (*Representative Concentration Pathway*) 8.5 (Gattuso et al., 2015).

The impact of ocean acidification and warming on single species has already been largely examined (Kroeker et al., 2013; Hurd et al., 2009). This approach has been useful for understanding the response of organisms and the mechanisms set up to cope with future changes. Marine calcifiers are particularly vulnerable to ocean acidification (Azevedo et al., 2015), with a decrease in survival, growth and calcification rate both for calcareous algae (e.g. Campbell et al., 2016; McCoy and Kamenos, 2015) and invertebrates (e.g. Dupont et al., 2010; Parker et al., 2013). On the other hand, non-calcareous organisms such as fleshy macroalgae and seagrasses are likely to experience a direct increase in their photosynthesis and growth from the increase in CO_2 (Koch et al., 2013). Increasing temperature might also have major effects on organisms' physiology through changes in biochemical rates, which determine metabolic rates and energy expend (Kordas et al., 2011; Tagliarolo et al., 2013). Since ocean acidification will occur in combination with ocean warming, its impact on organisms may be additive or mitigative than the single factors effects (Connell and Russell, 2010). Therefore, considering the combined effects of ocean acidification and warming is critical for accurately forecasting organisms' responses to climate change.

Although the examination of organism response to climate change offers interesting prospects to understand the physiological response of organisms, a lack of accurate ecological representation persists (Hale et al., 2011). It seems obvious that any direct impact of climate change at the species level will lead to indirect effects at the community level through changes in species interactions (Lord et al., 2017). Understanding interaction mechanisms among species in marine communities is essential for a better prediction of ecosystem responses in a context of climate change (Gaylord et al., 2015). This community level approach is thus at the center of current concerns in climate change research. Among benthic communities, a growing number of multi-specific studies highlight an impact of ocean acidification and warming on interactions between calcareous and fleshy macroalgae (Hofmann et al., 2012; Olabarria et al., 2013; Short et al., 2015), macroalgae and grazers (Alsterberg et al., 2013; Falkenberg et al., 2014; Sampaio et al., 2017)

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https://doi.org/10.1016/j.marenvres.2018.02.010
Received 24 October 2017; Received in revised form 5 February 2018; Accepted 11 February 2018
Available online 12 February 2018

and predators and preys (Ferrari et al., 2011; Lord et al., 2017; Rall et al., 2010). Despite this growing interest, studies examining the effect of climate change on multi-species assemblages composed by coralline algae, fleshy macroalgae and grazers are scarce (Asnaghi et al., 2013, 2014). In addition to *in vitro* experiments, several *in situ* studies were conducted at shallow coastal sites where volcanic CO_2 vents lower the pH of the seawater (Hall-Spencer et al., 2008; Kroeker et al., 2011, 2012; Porzio et al., 2011). These researches provided important insights on how acidification may affect the structure and the functioning of marine ecosystems, through the removal of susceptible organisms.

Intertidal rock pools are highly productive habitats (Martins et al., 2007) and are comprised of an important diversity of seaweeds and invertebrates (Araujo et al., 2006; Firth et al., 2014; Ganning, 1971). They are the place of strong interactions between species, including herbivory and competition (Metaxas and Scheibling, 1993). Communities colonizing rock pools experience high daily and seasonal variations in physico-chemical parameters, especially temperature and pH (Metaxas et al., 1994; Morris and Taylor, 1983). Several studies suggest that the consequences of ocean acidification on species to fluctuating environments (seasonal and diurnal variations) were noticeably reduced compared to those on species from more stable habitat (Benedetti-Cecchi et al., 2006; Findlay et al., 2010; Pansch et al., 2014; Whiteley, 2011). Within rock pools, the response of communities to changes in temperature and pCO_2 have been addressed by Olabarria et al. (2013), who only considered interactions between macroalgal species. To our knowledge, no study assessed the effect of increased temperature and pCO_2 on rock pool assemblages composed by coralline algae, fleshy algae and grazers. As seasonal variations are likely to modify the response of species to ocean acidification and warming (Baggini et al., 2014; Godbold and Solan, 2013), it appears essential to consider seasonal fluctuations in experimental studies.

In this context, the current study examined – through a controlled laboratory experiment – the combined effect of ocean acidification and warming on the productivity of rock pool multi-specific assemblages composed by two coralline algal species (*Ellisolandia elongata* and *Lithophyllum incrustans*), three fleshy algal species (*Chondrus crispus*, *Ulva* sp. and *Bifurcaria bifurcata*) and two grazer species (*Patella ulyssiponensis* and *Steromphala pennanti*). The effects of ocean acidification and warming have been assessed at both individual and community levels to test the importance of species interactions in the response of assemblages. As natural communities are subjected to environmental variability due to emersion/immersion cycles, experimental organisms were submitted to diurnal fluctuations of physico-chemical parameters by simulating tidal cycles. We hypothesized that rock pool species and assemblages have physiological advantage when facing predicted ocean acidification and warming. This hypothesis was tested in both winter and summer conditions.

2. Materials and methods

2.1. Rock pool description

The main species colonizing mid-intertidal rock pools at the Bloscon site in Roscoff, Brittany, France (48°43′28N 03°58′08W) were collected on 11 January 2016 (winter conditions) and 01 August 2016 (summer conditions). All collected species were present throughout the year in mid-intertidal rock pools. These rock pools are characterized by high seasonal and diurnal variations in physico-chemical parameters Legrand et al. (2018). Temperature and pH variations may exceed 18 °C and 1.7 units, respectively, between winter and summer. Diurnal temperature and pH variations may reach 8 °C and 2.5 units, respectively, during the summer Legrand et al. (2018).

2.2. Biological material

Small fragments of the crustose coralline alga *Lithophyllum incrustans* Philippi, 1837 were removed from the substrate from a rock pool on the mid intertidal shore. Selected fragments were entirely pink and did not present any bleaching. The geniculate coralline alga *Ellisolandia elongata* Ellis and Solander, 1786, was also selected from rock pools and carefully removed from their substrate. Similarly, three fleshy algal species were sampled (the foliose green alga *Ulva* sp., the brown alga *Bifurcaria bifurcata* Ross, 1958, and the red alga *Chondrus crispus* Stackhouse, 1797). Additionally, the two gastropods *Patella ulyssiponensis* Gmelin, 1791 and *Steromphala pennanti* Philippi, 1846 were sampled. Individuals of the same species were collected in the same rock pool when possible. All selected species were present throughout the year within natural rock pools. Although their natural biomass varied according to seasons (Legrand et al., 2018), we maintained similar biomass between winter and summer experiments to keep the communities consistent between experiments. After collection, all species were transported in seawater tanks to the Biological Station of Roscoff.

2.3. Experimental set-up

Two three-month long laboratory experiments were conducted. The first experiment was performed from January to April 2016 in winter conditions and the second experiment from August to November 2016 in summer conditions. At each season, 12 g FW of *Ulva* sp. and *Bifurcaria bifurcata*, 8 g FW of *Chondrus crispus*, 4 g FW of *Ellisolandia elongata*, about 4 cm^2 of *Lithophyllum incrustans*, 2 individuals of *Patella ulyssiponensis* and 3 individuals of *Steromphala pennanti* were randomly assigned to 20 15-L aquaria. Algae were fixed on glass marbles using nylon wire and epoxy resin to keep them in the bottom of aquaria. Organisms were acclimated to laboratory conditions over 7 days at *in situ* temperature (winter: 10.4 °C; summer: 15.6 °C) and pH (winter: 7.98; summer: 8.08). At the beginning of the experiment, pH was gradually decreased over 7 days by 0.05 units per day. The pH was regulated by modifying pCO_2 through CO_2 bubbling (Gattuso and Lavigne, 2009). On the other hand, temperature was increased by 0.5 °C per day. At each season, two pCO_2 conditions were tested, crossed with two temperature conditions (supplementary material, S1). The following four treatments were obtained:

1) Ambient pCO_2 and ambient temperature (control, A-pCO_2; T)
2) High pCO_2 and ambient temperature (H-pCO_2; T)
3) Ambient pCO_2 and high temperature (A-pCO_2; T + 3 °C)
4) High pCO_2 and high temperature (H-pCO_2; T + 3 °C).

Ambient pH conditions (A-pCO_2) corresponded to *in situ* winter (7.98) and summer (8.08) mean pH_T (pH on the total scale) recorded at the Estacade site, in Roscoff, by SOMLIT (*Service d'Observation en Milieu Littoral* from 2010 to 2015). High pCO_2 (H-pCO_2) corresponded to the "business-as-usual" scenario predicted for the end of the century, with a pH decrease of −0.33 units (RCP 8.5; Gattuso et al., 2015). Ambient temperature (T) corresponded to *in situ* winter (10.4 °C) and summer (15.6 °C) mean conditions recorded at the Estacade site by SOMLIT (from 2010 to 2015). High temperature (T + 3 °C) was determined according to the "business-as-usual" scenario predicted for 2100, with an increase in temperature of 2.7 °C (Gattuso et al., 2015).

The pH and the temperature were adjusted in four 100 L header tanks, continuously supplied with filtered (5 µm) natural seawater pumped directly in front of the Biological Station of Roscoff. The water flow rate was of 150 L h^{-1} per tank. The temperature and the pH were controlled by an off-line feedback system (IKS Aquastar, Karlsbad, Germany) that activated or stopped heaters and solenoid valves, controlling temperature and CO_2 (Air Liquide, France) bubbling in the tanks, respectively. Each header tank supplied with seawater five 15-L aquaria. As natural rock pools are isolated from the sea at low tide, emersion/immersion cycles were simulated using pumps and timers over cycles of 6 h. Temperature and pH values were different between

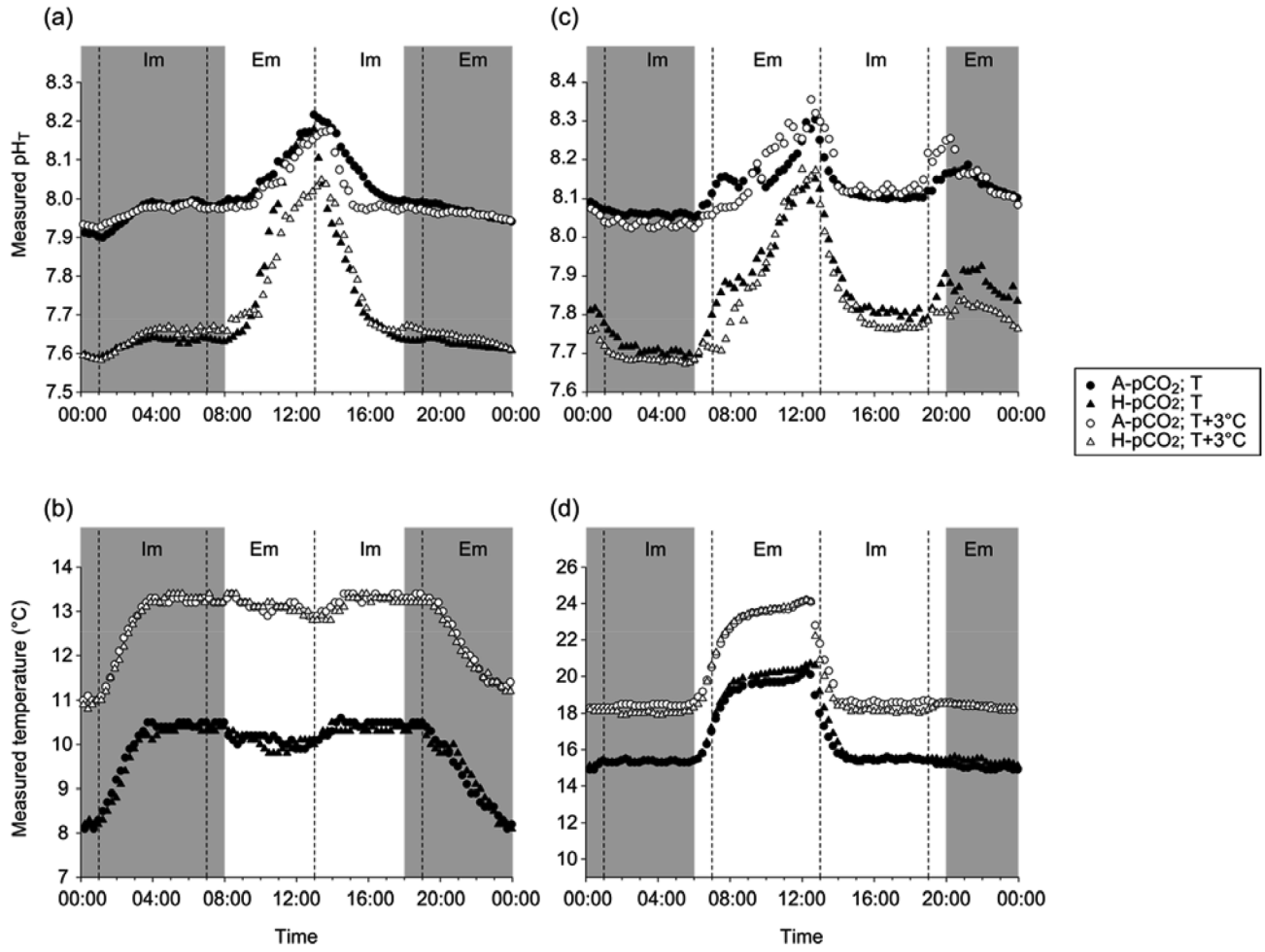

Fig. 1. Diurnal variations of pH_T and temperature simulating winter (a and b) and summer (c and d) emersion (Em)/immersion (Im) cycles for each pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatment. Gray sections represent the night. For each treatment, expected conditions of pH_T and temperature for the different emersion/immersion phases are shown at the top of each graph. The pH_T was only regulated during immersion phases.

immersion and emersion (Fig. 1).

During the immersion, aquaria were continuously supplied in seawater from header tanks using pumps, with a water flow rate of 15 L h^{-1} per aquarium. Temperature was maintained constant (winter: 10.4 °C and 13.4 °C; summer: 15.6 °C and 18.6 °C, for ambient and predicted temperature, respectively) in aquaria with water baths. Seawater pH (pH_T, expressed on the total hydrogen ion concentration scale, Dickson et al., 2007) and temperature were monitored every two days in the 20 aquaria, at different times of the day-time immersion. Seawater pH_T and temperature measurements were carried out using a pH probe combined with a temperature sensor (PHC101, Hach Lange, IntelliCAL). The pH probe was calibrated using Tris/HCl and 2-aminopyridine/HCl buffers (Dickson et al., 2007). The pH values of the offline feedback system were adjusted from measurements of pH_T carried out during immersion in each aquarium (Table 1). Total alkalinity (A_T) was also monitored throughout experiments in each aquarium during the day-time immersion (n = 30). For A_T analyses, seawater samples (60 mL) were filtered through 0.7 µm Whatman GF/F filters and

Table 1
Physical and chemical parameters (mean ± SE) of seawater measured during the immersion phase in each experimental condition (A-pCO_2 = ambient pCO_2; H-pCO_2 = high pCO_2; T = ambient temperature; T + 3 °C = high temperature) in the winter and the summer. pH_T and temperature were monitored every two days in each aquarium. Total alkalinity values (A_T) are means (± SE) of 30 samples measured in each aquarium. The CO_2 partial pressure (pCO_2), dissolved inorganic carbon (DIC), and saturation states of seawater with respect to aragonite (Ω_{Ar}) and calcite (Ω_{Ca}) were calculated from pH_T, temperature, salinity, and mean A_T using CO2SYS.

Season	Treatment	pCO_2 (µatm)	pH_T	% saturation O_2	Temperature (°C)	A_T (µmol kg^{-1})	DIC (µmol kg^{-1})	Ω_{Ar}	Ω_{Ca}
Winter	A-pCO_2; T	501 (± 11)	7.96 (± 0.01)	110.1 (± 0.7)	10.1 (± 0.1)	2386 (± 2)	2225 (± 4)	1.88 (± 0.03)	2.95 (± 0.05)
	H-pCO_2; T	1308 (± 25)	7.65 (± 0.01)	108.9 (± 2.2)	10.0 (± 0.0)	2373 (± 4)	2350 (± 4)	0.82 (± 0.01)	1.28 (± 0.02)
	A-pCO_2; T + 3 °C	440 (± 13)	7.96 (± 0.01)	112.4 (± 0.6)	13.3 (± 0.0)	2381 (± 3)	2173 (± 5)	2.33 (± 0.05)	3.65 (± 0.08)
	H-pCO_2; T + 3 °C	1179 (± 13)	7.65 (± 0.00)	110.2 (± 0.9)	13.3 (± 0.1)	2387 (± 2)	2333 (± 2)	1.04 (± 0.01)	1.62 (± 0.02)
Summer	A-pCO_2; T	401 (± 4)	8.07 (± 0.01)	103.7 (± 0.7)	15.7 (± 0.1)	2443 (± 2)	2193 (± 3)	2.80 (± 0.02)	4.34 (± 0.03)
	H-pCO_2; T	887 (± 20)	7.75 (± 0.01)	106.5 (± 1.0)	15.5 (± 0.1)	2437 (± 1)	2324 (± 5)	1.54 (± 0.03)	2.39 (± 0.05)
	A-pCO_2; T + 3 °C	430 (± 5)	8.04 (± 0.01)	102.6 (± 0.6)	18.6 (± 0.1)	2439 (± 1)	2178 (± 2)	2.95 (± 0.02)	4.55 (± 0.03)
	H-pCO_2; T + 3 °C	986 (± 12)	7.72 (± 0.01)	102.4 (± 0.8)	18.6 (± 0.1)	2435 (± 1)	2323 (± 2)	1.56 (± 0.02)	2.40 (± 0.03)

immediately poisoned with a mercuric chloride solution to prevent further biological activity (Dickson et al., 2007). A_T was determined using open-cell titration on an automatic titrator (Titroline alpha, Schott SI Analytics, Mainz, Germany) according to the method developed by Dickson et al. (2007). A_T was calculated using a Gran function applied to pH values ranging from 3.5 to 3.0 (Dickson et al., 2007) and corrected using standard reference material provided by the Andrew G. Dickson laboratory 125 (CRM Batch 111, accuracy of ± 6 µmol kg^{-1}). Salinity was measured every 2 weeks with a conductivity probe (CDC401, Hach Lange, IntelliCAL, accuracy of 0.1) and remained constant during experiments (winter: 35.1 ± 0.1; summer: 35.3 ± 0.1). Irradiance during immersion was set to the mean *in situ* irradiance measured at the Station Biologique de Roscoff and corrected from an extinction coefficient of light in seawater at 3 m depth (mean marnage). Light intensities were 40 µmol photons m^{-2} s^{-1} in the winter and 100 µmol photons m^{-2} s^{-1} in the summer. The light was provided by two or four 80 W fluorescent tubes (JBL Solar Ultra Marin Day, JBL Aquaria, Nelson, New Zealand) above the aquaria under a 10/14 h or 14/10 h light/dark photoperiod, for winter or summer conditions, respectively.

During the emersion, seawater supply to the aquaria was stopped by deactivating pumps. Therefore, the pH in each aquarium only varied according to the community metabolism. The temperature in aquaria was maintained to reflect mean *in situ* temperatures measured during night- (winter: 8.0 °C; summer: 14.0 °C) and day-time emersion (winter: 10.0 °C; summer: 20.2 °C) (Legrand et al., submitted; Fig. 1) and predicted conditions for the end of the century (winter: night = 11.0 °C, day = 13 °C; summer: night = 17.0 °C, day = 23.2 °C). Similarly, irradiance during day-time emersion was set to the mean *in situ* irradiance previously measured above rock pools: 90 µmol photons m^{-2} s^{-1} in the winter and 200 µmol photons m^{-2} s^{-1} in the summer.

2.4. Metabolic measurements

At the end of the winter and the summer laboratory experiments, metabolic measurements were conducted both on species in isolation and on whole assemblages. Incubations were performed in acrylic respirometry chambers (Engineering and Design Plastics Ltd, Cambridge, UK). The chamber volume depended on species size and was of 185 mL for *P. ulyssiponensis, S. pennanti*, *E. elongata*, *L. incrustans, C. crispus* and *B. bifurcata* and 600 mL for *Ulva* sp. and assemblages. Species were incubated with the seawater of each aquarium. During incubations, species were placed on a plastic grid above a stir bar in the chambers to ensure the seawater was well mixed. For the gastropods, respiration rates were measured under ambient irradiance. For each grazer species, individuals present in each aquarium were incubated together. For the algal species and assemblages, net photosynthetic rates were measured under ambient irradiance, and respiration rates were measured in the dark. For light incubations, chambers were placed inside aquaria to control temperature. For dark incubations, chambers were placed in a plastic crate filled with aquaria seawater in an open circuit to keep the temperature constant. Incubation duration was adjusted to keep oxygen saturation above 80%. Incubations lasted approximately from 1 h for *P. ulyssiponensis* to 2 h for *L. incrustans*. For assemblages, the metabolism was measured from the incubations of all species together.

Oxygen concentrations were measured at the beginning and at the end of each incubation, using an optical fiber system (FIBOX 3, PreSens, Regensburg, Germany). Reactive spots were calibrated with 0% and 100% buffer solutions. The 0% buffer solution was prepared by dissolving 1 g of sodium sulfite (Na_2SO_3) in 100 mL of seawater. The 100% buffer solution was prepared by bubbling air into 100 mL of seawater using an air-pump for 20 min to obtain air-saturated seawater. Net primary production (NPP, µmol O_2 g DW^{-1} h^{-1}) or respiration (R, µmol O_2 g DW^{-1} h^{-1}) rates were calculated following Eq. (1):

$$\text{NPP or R} = \frac{\Delta O_2 \times V}{\Delta t \times DW} \quad (1)$$

where ΔO_2 is the difference between the initial and final oxygen concentrations (µmol O_2 L^{-1}), V, the volume of the chamber (L), Δt, the incubation time (h), and DW, the dry weight of the species incubated (g). The dry weight was obtained after 48 h at 60 °C. For gastropods, the body was separated from the shell to consider the dry weight of the body only.

For the algae and the assemblages, gross primary production (GPP) was calculated following Eq. (2):

$$\text{GPP} = \text{NPP} + |\text{R}| \quad (2)$$

Control incubations containing only seawater were carried out to correct for oxygen fluxes due to any additional biological activity in seawater. Oxygen fluxes calculated in control chambers were subtracted from oxygen fluxes of chambers containing species.

2.5. Crustose coralline algae (CCA) bleaching measurement

At the end of each three-month long experiment, all incubated thalli of *L. incrustans* were photographed. The bleaching is characterized by the presence of white patches at the surface of *L. incrustans* thalli. The percentage of bleached surface was determined using Image J software version 1.46 (Rasband, 2016).

2.6. Chlorophyll a analysis

At the end of the experiments, thalli of all algae were collected in each aquarium and immediately frozen at −20 °C pending analyses. Then samples were freeze-dried and crushed into a powder using a mortar, in the dark. An aliquot of 0.02–0.10 g of powder was precisely weighed and suspended in 10 mL of 90% acetone and stored in the dark at 4 °C for 12 h. Samples were then centrifuged at 4000 rpm. The supernatant was collected and absorbance was measured at 630, 647, 664, and 691 nm. Chlorophyll *a* (Chl *a*) concentrations (µg g DW^{-1}) were calculated from Ritchie (2008).

2.7. Data analysis

Statistical analyses were carried out using the statistical software R, version 3.2.2. To examine the importance of species interactions in the response of the community to predicted changes, the metabolism measured for assemblages was compared to the metabolism expected for assemblages if no interaction occurred between species. Expected metabolism was calculated from the sum of individual fluxes obtained from specific incubations (Tait and Schiel, 2011). Mean measured and expected metabolic rates of assemblages (gross primary production and respiration rates) were compared using Wilcoxon rank sum tests.

The influence of temperature (two levels: ambient and elevated temperature) and pCO_2 (two levels: ambient and elevated pCO_2) was tested on metabolic rates of grazers, coralline and fleshy algae and assemblages (measured and expected metabolic rates) using two-way non-parametric Scheirer-Ray-Hare tests (n = 5). Winter and summer conditions were then compared using a Wilcoxon rank sum test for each variable.

3. Results

3.1. Seasonal effect

Respiration rates (R) of the gastropods *P. ulyssiponensis* and *S. pennanti* exhibited a strong seasonal pattern, with higher R recorded in the summer than in the winter (Table 2). In *E. elongata*, *C. crispus* and *B. bifurcata*, higher gross primary production (GPP) and R were recorded in the winter than in the summer (Table 2). GPP and R in *L. incrustans*,

Table 2
Results of Wilcoxon rank sum tests testing the differences in GPP, R and chlorophyll *a* content (for seaweeds) of the different species and the assemblages between winter and summer experiments (n = 20). Significant values (α < 0.05) are in bold.

	Gross primary production GPP		Respiration R		Chlorophyll a	
	W	p	W	p	W	p
P. ulyssiponensis			50	< **0.001**		
S. pennanti			39	< **0.001**		
E. elongata	27	< **0.001**	307	**0.003**	29	< **0.001**
L. incrustans	216	0.678	207	0.862	92	**0.017**
C. crispus	109	**0.013**	361	< **0.001**	11	< **0.001**
Ulva sp.	244	0.242	169	0.413	3	< **0.001**
B. bifurcata	59	< **0.001**	305	**0.004**	29	< **0.001**
Assemblage measured fluxes	217	0.659	136	0.086		
Assemblage expected fluxes	250	0.183	105	**0.010**		

Ulva sp. and assemblages were not significantly affected by the season. For all algal species, chlorophyll *a* content was significantly higher in the winter than in the summer (Tables 2 and 3). In *L. incrustans*, bleaching was significantly higher in the summer (Wilcoxon rank sum test, W = 328, p < 0.001).

3.2. Algal chlorophyll a content and bleaching

Chlorophyll *a* content presented differences between species, with the highest values recorded in *B. bifurcata* and the lowest values measured in *L. incrustans* (Table 3). Increased temperature and pCO_2 did not affect chlorophyll *a* content in *C. crispus* and *B. bifurcata* regardless of the season. In *Ulva* sp., chlorophyll *a* content did not differ among temperature and pCO_2 treatments in the winter, while a significant increase was detected under elevated temperature in the summer. In *E. ellisolandia* and *L. incrustans*, no significant effect of increased temperature and pCO_2 on chlorophyll *a* content was detected in the winter, while an interactive effect of these two factors was evidenced in the summer (Table 4).

Bleached surface was observed in *L. incrustans* in all temperature and pCO_2 treatments. The percentage of bleaching was significantly higher under elevated temperature and pCO_2 in the winter, while no effect was evidenced in the summer (Fig. 2; Table 5).

3.3. Productivity and respiration of species

No effect of elevated temperature and pCO_2 was observed on *P. ulyssiponensis* R (Fig. 3; Table 4), while *S. pennanti* R was enhanced under elevated temperature in winter conditions. In coralline algae, the response to ocean acidification and warming is species-specific. In *E. elongata*, GPP significantly increased under elevated temperature in the winter, and decreased under high pCO_2 in the summer (Fig. 4; Table 4). When combined, increased temperature and pCO_2 have a synergistic effect on *E. elongata* R in the winter, as increased pCO_2 enhanced R under elevated temperature only. Conversely, in the summer, R was lower when increased pCO_2 was combined with increased temperature. GPP and R in *L. incrustans* were not affected by elevated temperature and pCO_2 in the summer. In the winter, increased pCO_2 and temperature have an antagonistic effect on *L. incrustans* GPP and R (Fig. 4; Table 4).

A significant interactive effect of temperature and pCO_2 was detected on *C. crispus* GPP in the winter. In the summer, an increase in GPP was observed under high pCO_2 (Fig. 5; Table 4). *C. crispus* R was significantly higher under elevated pCO_2 and temperature conditions in the winter and the summer, respectively. GPP in *Ulva* sp. did not vary among temperature and pCO_2 conditions regardless of the season (Fig. 5; Table 4). In winter conditions, an antagonistic effect of temperature and pCO_2 was observed on *Ulva* sp. R. After three months in summer conditions, an increase in R was observed under elevated temperature, while a decrease in R was observed under high pCO_2. In the winter, *B. bifurcata* GPP was significantly reduced under elevated temperature, while no effect of temperature or pCO_2 was detected in the summer (Fig. 5; Table 4). A decline in *B. bifurcata* R was evidenced under elevated temperature in the winter, while an increase in R was detected in the summer.

3.4. Productivity and respiration of assemblages

Expected GPP was significantly higher than measured GPP both in the winter and the summer (Wilcoxon rank sum tests, p-value < 0.001). A significant difference was also detected between measured and expected R in the winter (p-value = 0.021), while no difference was observed in the summer (p-value = 0.99). Assemblage GPP increased under elevated temperature in the summer (Fig. 6; Table 4). Measured R was significantly affected by the interaction between temperature and pCO_2 in the winter, while no effect was observed in the summer. Assemblages expected R was significantly higher in the summer, while no effect of season was detected on expected GPP.

Table 3
Chlorophyll *a* content (mean ± SE, n = 5) of calcareous and fleshy algae in the different pCO_2 (A-pCO_2 = ambient pCO_2; H-pCO_2 = high pCO_2) and temperature (T = ambient temperature; T + 3 °C = high temperature) treatments, after being maintained three months in winter and summer conditions.

	Chlorophyll *a* µg chlorophyll g DW^{-1}			
	A-pCO_2/T	H-pCO_2/T	A-pCO_2/T + 3 °C	H-pCO_2/T + 3 °C
Ellisolandia elongata				
Winter	51.2 (± 10.7)	68.2 (± 10.6)	50.0 (± 10.1)	52.0 (± 4.5)
Summer	28.3 (± 3.2)	33.3 (± 2.6)	28.9 (± 0.9)	21.7 (± 2.3)
Lithophyllum incrustans				
Winter	22.8 (± 2.8)	18.9 (± 3.6)	23.4 (± 3.6)	15.8 (± 1.7)
Summer	13.0 (± 1.1)	12.3 (± 3.0)	18.7 (± 1.3)	15.4 (± 1.5)
Chondrus crispus				
Winter	34.6 (± 6.1)	30.1 (± 4.8)	31.6 (± 3.9)	31.5 (± 2.2)
Summer	17.1 (± 1.6)	17.5 (± 4.8)	17.7 (± 2.3)	14.7 (± 1.7)
***Ulva* sp.**				
Winter	133.8 (± 14.4)	152.9 (± 12.0)	123.1 (± 13.1)	127.9 (± 10.6)
Summer	41.9 (± 4.6)	44.0 (± 7.2)	77.7 (± 11.3)	58.4 (± 9.1)
Bifurcaria bifurcata				
Winter	218.9 (± 12.0)	210.9 (± 7.2)	213.3 (± 16.7)	205.6 (± 19.2)
Summer	129.7 (± 17.1)	146.7 (± 10.4)	159.0 (± 14.1)	168.4 (± 9.0)

Table 4
Results of the two-way non-parametric Scheirer-Ray-Hare tests for the effects of temperature (T) and pCO_2 on the respiration rates of species and assemblages (n = 5). Significant p-values are shown in bold (α = 0.05). ↗: increase in metabolic rates with increased temperature or pCO_2; Degree of freedom = 1. Significant values (α < 0.05) are in bold.

			Gross production GPP		Respiration R		Chlorophyll a		Bleaching	
			F	p-value	F	p-value	F	p-value	F	p-value
P. ulyssiponensis	**Winter**	T			3.0	0.082				
		pCO_2			0.7	0.406				
		T x pCO_2			1.3	0.257				
	Summer	T			2.8	0.096				
		pCO_2			0.1	0.705				
		T x pCO_2			1.5	0.226				
S. pennanti	**Winter**	T			5.9	**0.016** ↗				
		pCO_2			0.1	0.821				
		T x pCO_2			1.9	0.174				
	Summer	T			1.5	0.226				
		pCO_2			0.1	0.705				
		T x pCO_2			0.2	0.650				
C. crispus	**Winter**	T	0.0	0.934	0.4	0.545	0.1	0.762		
		pCO_2	0.1	0.705	7.4	**0.006** ↗	0.0	0.940		
		T x pCO_2	7.0	**0.008**	1.7	0.199	0.1	0.762		
	Summer	T	0.6	0.450	4.8	**0.028** ↗	0.0	0.940		
		pCO_2	5.1	**0.023** ↗	1.9	0.174	0.8	0.364		
		T x pCO_2	2.5	0.112	0.0	0.999	0.8	0.364		
Ulva sp.	**Winter**	T	0.7	0.406	0.0	0.880	1.3	0.257		
		pCO_2	2.5	0.112	4.5	**0.034**	1.5	0.226		
		T x pCO_2	0.7	0.406	7.8	**0.005**	0.1	0.762		
	Summer	T	0.4	0.545	4.5	**0.034** ↗	7.4	**0.006** ↗		
		pCO_2	0.2	0.650	7.4	**0.006** ↘	0.3	0.597		
		T x pCO_2	0.7	0.406	0.3	0.597	1.9	0.174		
B. bifurcata	**Winter**	T	192.2	**0.019** ↘	10.1	**0.001** ↘	0.1	0.705		
		pCO_2	28.8	0.364	0.3	0.597	1.0	0.326		
		T x pCO_2	28.8	0.364	0.2	0.650	0.0	0.940		
	Summer	T	2.5	0.112	8.3	**0.004** ↗	2.8	0.096		
		pCO_2	0.5	0.496	0.3	0.597	1.3	0.257		
		T x pCO_2	0.1	0.705	2.3	0.131	0.1	0.762		
E. elongata	**Winter**	T	12.6	< **0.001** ↗	7.8	**0.005**	1.1	0.290		
		pCO_2	1.9	0.174	0.4	0.545	0.8	0.364		
		T x pCO_2	0.2	0.650	5.1	**0.023**	0.7	0.406		
	Summer	T	2.1	0.151	0.8	0.364	2.1	0.151		
		pCO_2	5.1	**0.023** ↘	5.5	**0.019**	0.2	0.650		
		T x pCO_2	2.8	0.096	5.9	**0.016**	6.6	**0.010**		
L. incrustans	**Winter**	T	1.3	0.257	2.8	0.096	0.5	0.496	3.9	**0.049** ↗
		pCO_2	0.6	0.450	0.0	0.940	3.0	0.082	4.0	**0.045** ↗
		T x pCO_2	9.1	**0.002**	14.3	< **0.001**	0.1	0.705	0.0	0.910
	Summer	T	1.0	0.326	2.5	0.112	2.3	0.131	1.3	0.257
		pCO_2	1.0	0.326	0.6	0.450	0.0	0.940	1.1	0.290
		T x pCO_2	0.5	0.496	0.2	0.650	4.5	**0.035**	1.1	0.290
Assemblage measured fluxes	**Winter**	T	0.4	0.545	0.4	0.545				
		pCO_2	0.1	0.762	4.2	**0.041** ↘				
		T x pCO_2	1.7	0.199	2.8	0.096				
	Summer	T	3.5	0.082	4.8	**0.028** ↗				
		pCO_2	0.3	0.586	1.9	0.174				
		T x pCO_2	0.5	0.493	2.3	0.131				
Assemblage expected fluxes	**Winter**	T	1.5	0.226	5.5	**0.019** ↘				
		pCO_2	0.7	0.406	1.3	0.257				
		T x pCO_2	0.1	0.762	0.5	0.496				
	Summer	T	0.1	0.762	0.8	0.364				
		pCO_2	0.0	0.999	2.1	0.151				
		T x pCO_2	2.1	0.151	3.3	0.070				

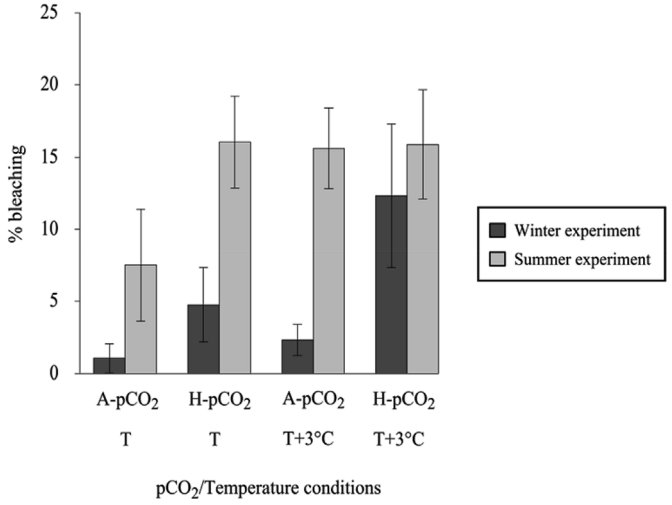

Fig. 2. Percentage of bleaching (mean ± SE, n = 5) in *Lithophyllum incrustans* in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments, after the three-month experiments in winter (dark gray) and summer (light gray) experiments.

Expected GPP did not differ among pCO_2 conditions regardless of the season, while R decreased under elevated temperature in the winter (Fig. 6; Table 4).

4. Discussion

4.1. Assemblage measured and expected metabolism

Expected metabolism simulates the potential response of the assemblage in the absence of interaction between species. Significant differences were observed between measured and expected GPP, while no difference was detected on R. These results are consistent, given that species interact for access to light and nutrients during the day. Within marine environments, species interactions are critical, driving the mechanisms for ecosystem persistence or loss under environmental pressure (Connell and Ghedini, 2015; Falkenberg et al., 2015). Within rock pools, the canopy structure has a major influence on the productivity of macroalgal assemblages (Bruno et al., 2005; Tait and Schiel, 2011). In this study, the presence of canopy species, such as *B. bifurcata*, may reduce incident light and thus the photosynthesis of understory species. Conversely, during day-time emersion, canopy species protect underlying species from the effects of high irradiances (Bulleri et al., 2002; Tait and Schiel, 2011). Although this study did not assess the metabolic response of species during the experimental emersion period, our results suggest the importance of considering species interactions in order to have a better overview of the response of rock pool communities to climate change.

4.2. Ocean acidification and warming effects on rock pool assemblages

Rock pools are generally considered as productive systems due to the high macroalgal biomass and high photosynthetic rates of seaweeds (Araujo et al., 2006; Björk et al., 2004; Metaxas et al., 1994; Morris and Taylor, 1983). In the present study, increased pCO_2 did not affect assemblage GPP regardless of the season, while elevated temperature increased GPP in the summer only. Assemblage GPP was mainly driven by the response of fleshy macroalgae to acidification and warming, although the three species examined in this study exhibited different responses to temperature and pCO_2 changes. The species-specific response of macroalgae to climate change has already been evidenced in several studies (Beardall et al., 1998; Gao et al., 1991; Kram et al., 2016; Middelboe and Hansen, 2007; Swanson and Fox, 2007). Mechanisms governing the physiological response of marine macroalgae to increased pCO_2 remain poorly documented (Hurd et al., 2009); however, it is likely that their response to predicted changes will depend on the mechanisms used for inorganic carbon uptake (Cornwall et al., 2017b).

Ulva sp. GPP was not affected by increased temperature and pCO_2 regardless of the season. Several studies evidenced the ability of *Ulva* sp. to use different forms of inorganic carbon through presence of powerful carbon concentration mechanisms (CCM; Axelsson et al., 1999; Björk et al., 2004; Rautenberger et al., 2015). This process is likely to provide a competitive advantage to *Ulva* sp., especially when CO_2 is limited (Axelsson et al., 1995). Similarly, *B. bifurcata* exhibited weak changes in photosynthesis under predicted conditions, except for a decrease in GPP under elevated temperature in the winter. *C. crispus* GPP was more sensitive to changes in temperature and pCO_2 alone, although the combined effect of increased temperature and pCO_2 moderated the shifts induced by single factors. In the winter, a decline in assemblage R was evidenced under predicted temperature and pCO_2 conditions. Among seaweeds, R was more sensitive to increased temperature and pCO_2 than GPP, which suggests that the response in terms of photosynthesis is not always related to respiration. In their study, Zou et al. (2011) suggested the link between algal respiration, photosynthesis and chlorophyll content, while Olabarria et al. (2013) evidenced that rock pool macroalgal photosynthesis and respiration were decoupled when temperature and pCO_2 increased. Our results are consistent with this last hypothesis.

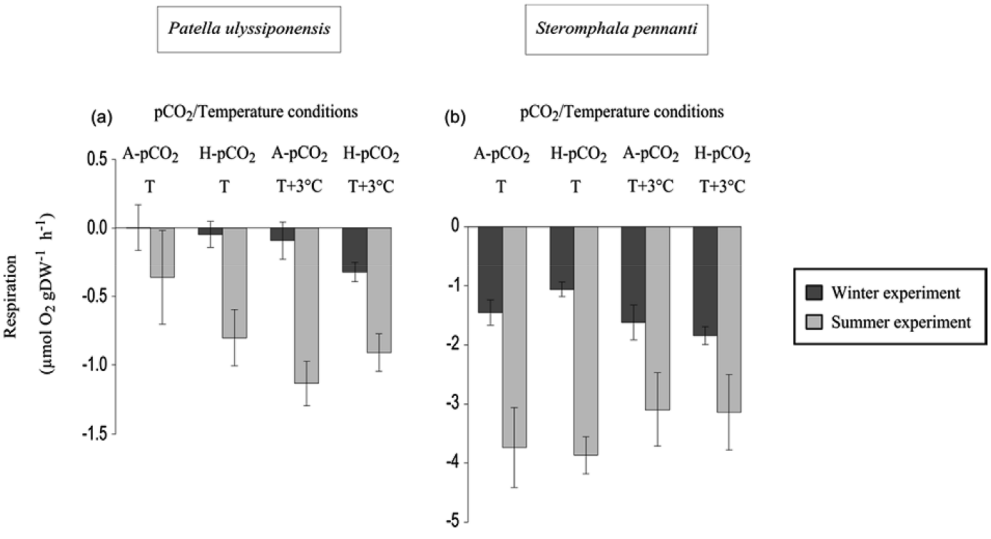

Fig. 3. Respiration rates (mean ± SE, n = 5) of the grazers (a) *Patella ulyssiponensis* and (b) *Steromphala pennanti* in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) conditions. The species were maintained in assemblages for three months in winter (dark gray) and summer conditions (light gray).

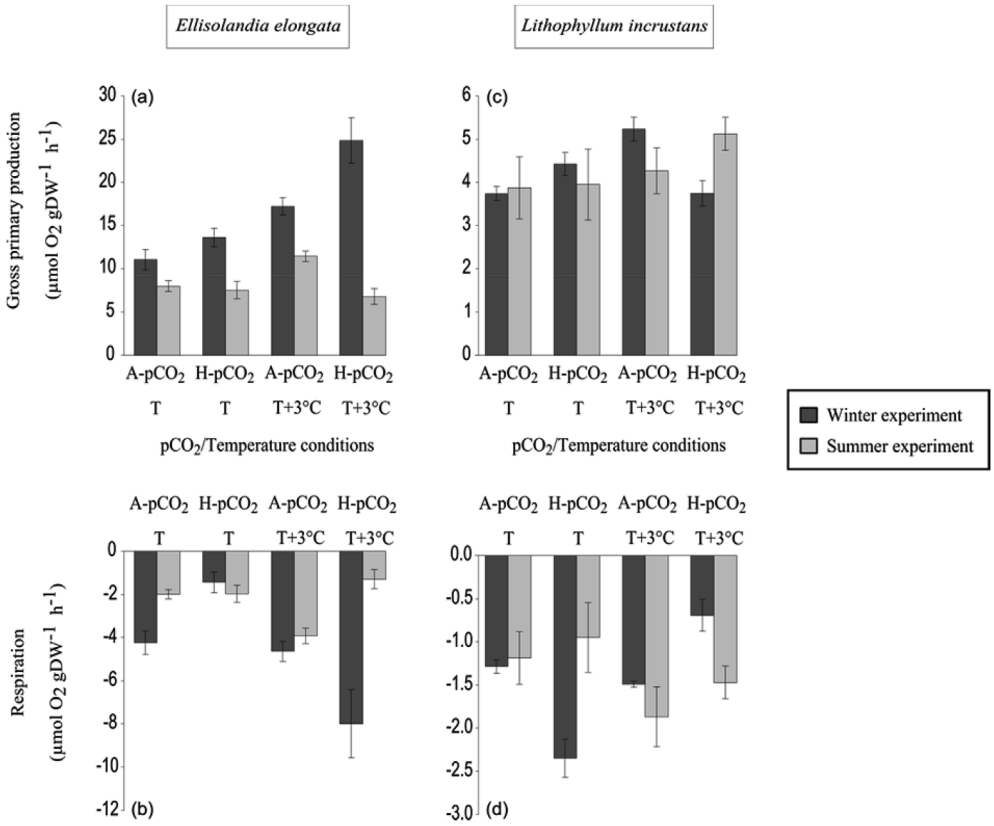

Fig. 4. Gross primary production and respiration rates (mean ± SE, n = 5) of *Ellisolandia elongata* (a,b) and *Lithophyllum incrustans* (c,d) in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T+3 °C = High temperature) treatments, after three months in winter (dark gray) and summer conditions (light gray).

Although coralline algae did not appear to strongly influence the response of experimental assemblages to acidification and warming, their response is likely to be more important within coralline dominated rock pools (Araujo et al., 2006; Legrand et al., 2018). As for fleshy algae, the photosynthetic and respiratory response of coralline algae to acidification and warming were species-specific. Most significant changes were observed on *E. elongata* GPP, which responded positively to increased temperature in the winter only. In the winter, the increase in R under combined elevated temperature and pCO_2 may be related to the change in GPP, due to the increase in the supply of respiratory substrates from photosynthesis (Egilsdottir et al., 2013; Semesi et al., 2009). The positive effect of increased temperature on coralline photosynthesis has already been reported in several studies (Martin et al., 2013), while pCO_2 effects on coralline photosynthetic rates are more contrasted (McCoy and Kamenos, 2015; Hofmann and Bischof, 2014). The photosynthetic response of coralline algae to ocean acidification depends on the mechanisms set up for inorganic carbon uptake (Cornwall et al., 2017a), which may vary within the same species according to the season. Indeed, increased pCO_2 did not affect *E. elongata* GPP in the winter, while GPP was significantly reduced in the summer. In their study, Egilsdottir et al. (2013) also evidenced that *E. elongata* photosynthesis was little affected by increased pCO_2 when maintained at low irradiances (30 µmol photons m^{-2}). Vasquez-Elizondo and Enriquez (2016) suggested that differences in light intensity may considerably modify the response of coralline algae to acidification and warming. Therefore, lower light intensities in winter conditions appeared more favorable for *E. elongata* photosynthesis (Williamson et al., 2014). This species is generally adapted to lower light intensities and colonize under-canopy environments or deeper and sheltered rock pools (Egilsdottir et al., 2016; Korbee et al., 2014). In *L. incrustans*, weak changes in photosynthesis and respiration were detected under predicted ocean acidification and warming. Indeed, the increase in GPP observed under elevated temperature in the winter was reduced when this factor was combined with elevated pCO_2. Conversely, *L. incrustans* bleaching was significantly higher under high temperature and pCO_2 conditions in the winter. Several studies also evidenced the impact of increased temperature and pCO_2 on crustose coralline algal bleaching (Anthony et al., 2008; Diaz-Pulido et al., 2012; Martin and Gattuso, 2009; Noisette et al., 2013). Other factors, such as diseases and pathogens, may have caused algal bleaching, as evidenced for tropical crustose coralline algae (Littler and Littler, 1998). In the summer higher bleaching was observed than in winter conditions. It is likely that summer light conditions may have caused damages to cell tissues, inducing bleaching of the thalli. Noisette et al. (2013) suggested that this negative impact of high irradiances may be amplified at high pCO_2 conditions. Our results do not support this statement, as no temperature or pCO_2 effect was detected in summer conditions on *L. incrustans* bleaching.

The relationship between seaweeds and calcareous macroalgae is critical in rock pool environments. By their high photosynthetic rates, seaweeds reduce the carbon dioxide (CO_2) concentration in the rock pools, making the environment more conducive to calcification for calcareous species (Legrand et al., 2018). Maintaining seaweed productivity under predicted acidification and warming is likely to reduce the impact of these factors on understory species. In addition, in natural ecosystems, grazers are often associated with an important ecological function, exerting a control on the composition and abundance of plant biomass (Hairston et al., 1960; Guillou et al., 2002; Bonaviri et al., 2011). In their study, Tagliarolo et al. (2013) evidenced the influence of temperature on the metabolism of intertidal gastropods. These results are in agreement with the increase in R detected in *S. pennanti* in the winter, but contrast with the lack of response observed in *P. ulyssiponensis* under elevated temperature. *P. ulyssiponensis* is considered as a micrograzer and its influence on macroalgal productivity is likely to be limited. In *S. pennanti*, R increase under high temperature in winter conditions did not seem to have implication on assemblage

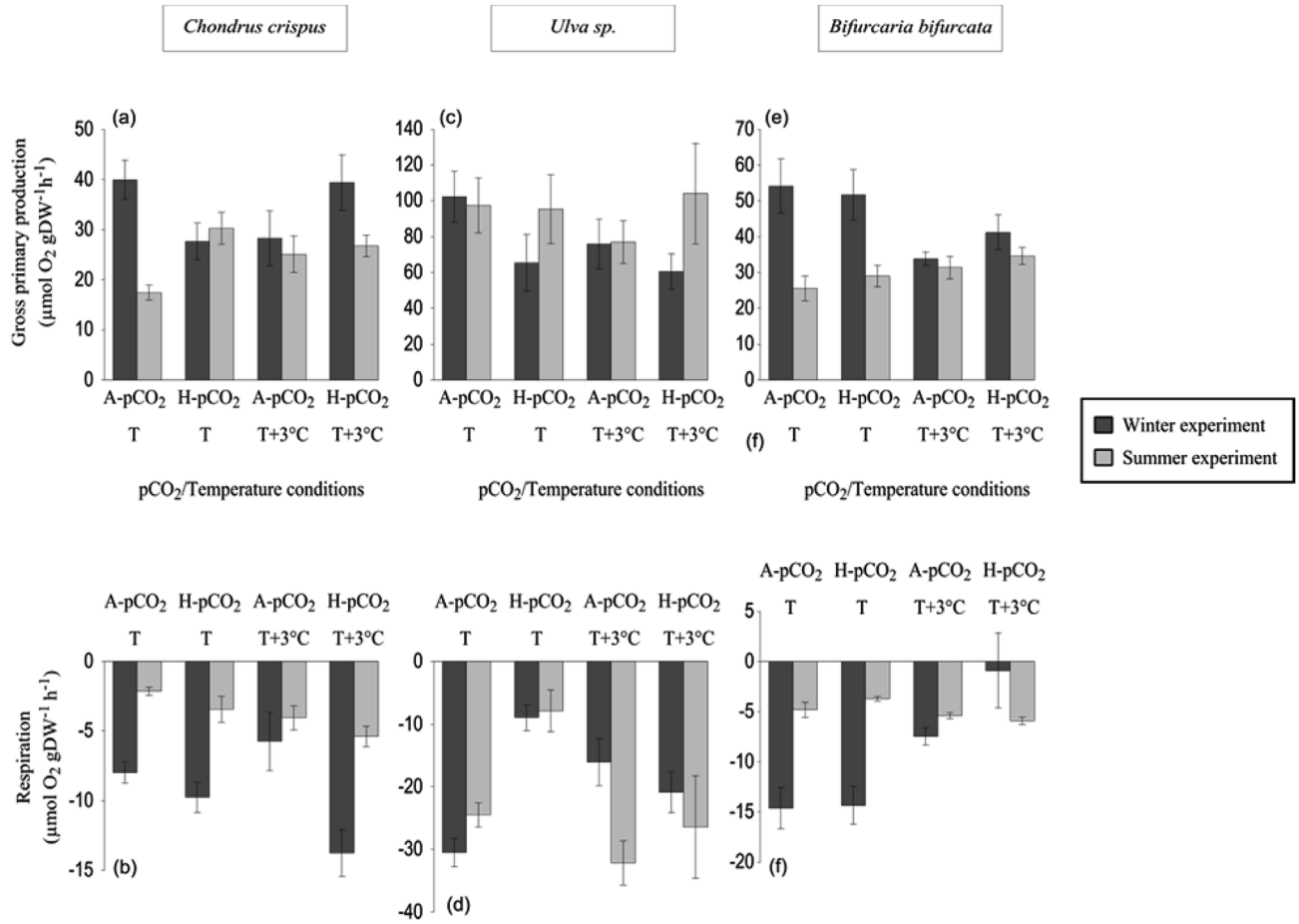

Fig. 5. Gross primary production and respiration rates (mean ± SE n = 5) of the fleshy algae *Chondrus crispus* (a,b,respectively), *Ulva* sp. (c,d) and *Bifurcaria bifurcata* (e,f) in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments. Species were maintained during three months in winter (dark gray) and summer (light gray) conditions.

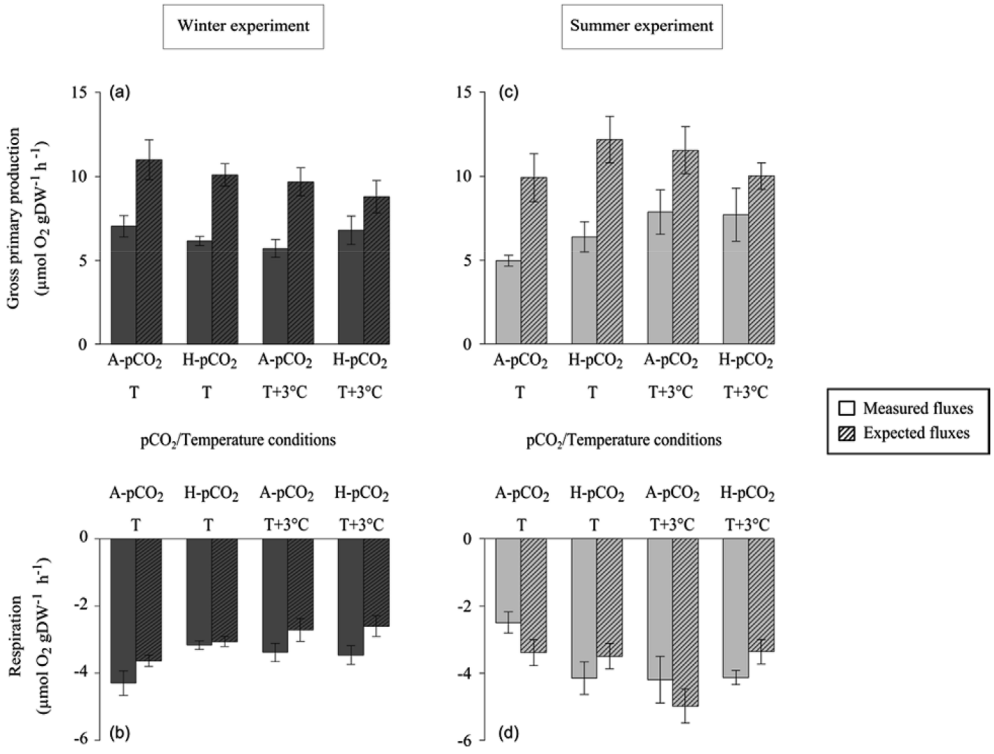

Fig. 6. Measured (full color) and expected (hatched) gross primary production and respiration rates (mean ± SE, n = 5) of assemblages in the different pCO_2 (A-pCO_2 = Ambient pCO_2; H-pCO_2 = High-pCO_2) and temperature (T = Ambient temperature; T + 3 °C = High temperature) treatments. The assemblages were maintained during three months in winter (dark gray; a,b) and summer conditions (light gray; c,d).

productivity, suggesting a negligible effect of this species at the community scale.

In conclusion, the present results evidenced that rock pool assemblages appear relatively robust to changes in temperature and pCO_2, in terms of primary production. The photosynthetic response of rock pool assemblages is likely to be mainly governed by the response of fleshy macroalgal species, which are little affected by predicted changes. Responses of rock pool species to predicted changes are species-specific and are likely to depend on the adaptation mechanisms they set up to cope with the natural physic-chemical variability of their habitat. For example, organisms colonizing rock pools higher on the shore experience greater variations (e.g. light, temperature, pH, oxygen) than rock pools lower on the shore (Morris and Taylor, 1983) and are likely to be more resistant to climate change. To test this hypothesis, we suggest that further experiments should be performed on rock pool assemblages, focusing on communities at different shore height and including metabolic measurement over complete tidal cycles (emersion and immersion conditions).

Conflicts of interest

None.

Acknowledgments

The authors thank the CRBM (Center of Marine Biologic Resources) at the Station Biologique de Roscoff for its kind permission to use their premises for the duration of the experiments. The authors thank Stéphane Loisel for his field assistance. We thank Chanel Duncan for language editing of the manuscript. This work was supported by the Brittany Regional Council and the French National Research Agency via the “Investment for the Future” program IDEALG (n° ANR-10-BTBR-04).

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.marenvres.2018.02.010.

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